Please enter new claims 15-18:

15. The method as claimed in claim 1, wherein the specific binding assay is an immunoassay.

16. The method as claimed in claim 1, wherein the specific binding assay is a receptor binding assay.

17. ~~The method as claimed in claim 1, wherein the assay reagents comprise a tracer.~~

18. The method as claimed in claim 1, wherein the assay reagents comprise a labeled assay reagent for detection wherein the label is selected from the group consisting of radioactive isotope labels, enzyme-linked labels and fluorescent labels.

REMARKS

I. Preliminary Remarks

Submitted herewith are amendments to Applicant's claims in accordance with the suggestions of the Examiner to address the various rejections under 35 U.S.C. §112 (second paragraph). Further, new method claims 15-18 are entered as well. In addition, the abstract was amended to incorporate the proper language and format consistent with 37 C.F.R. §1.72(b) and M.P.E.P. §608.01(b). The subject matter of the amendment is present in the application as filed and no new matter is introduced thereby.

II. Supplemental Information Disclosure Statement

Submitted herewith is a supplemental Information Disclosure Statement and a Form-1449. The Applicants respectfully request that this document (A6) be made of record and considered for the above-identified application. A copy of document A6 and the accompanying fee according to 37 C.F.R. §1.197(c) are also submitted herewith.

III. Claimed Subject Matter

The claims as amended relate to a method for detecting an analyte in a single reaction vessel wherein the cells are lysed by a detergent in the presence of a sequestrant, the analyte is detected with a scintillation proximity assay (SPA) or an immunoassay utilizing labeled antibodies. Claims 1-4 are directed toward the components of the assay where the cell lysis reagent is a detergent and the sequestrant is a cyclodextrin. Claims 5-7 are directed toward the assay being performed in a single reaction vessel on a multiwell plate where the cells are lysed and assayed in the same vessel. Claims 8-13 are directed toward the method of analyte detection and the types of analyte measured. Claim 14 is directed toward a kit to utilize this method for measuring an analyte. Claims 15-18 are directed toward types of assays embodied by the invention.

IV. Outstanding Rejections

Claims 1-14 stand rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to distinctly claim the subject matter of the invention.

Claims 1-4, 8-11, and 13-14 stand rejected under 35 U.S.C. §103(a) as being obvious over Cook (Research Focus 1(7): 287-94, 1996) (hereinafter "Cook (1)") in view of Lundin *et al.* (U.S. 5,558,986) (hereinafter "Lundin").

Claims 1-14 also stand rejected under U.S.C. §103(a) as being unpatentable over Cook (1) in view of Lundin, and in further view of Cook (WO 94/26413) (hereinafter "Cook (2)").

V. Patentability Arguments

A. The Rejection Under 35 U.S.C. §112, Second Paragraph Should be Withdrawn

The rejection of claims 1-14 under 35 U.S.C. §112, second paragraph, may properly be withdrawn in light of the foregoing amendments made to more clearly recite the subject matter of the invention.

Claims 1(i) is amended herein to clearly state the terms as described in the specification. The Examiner considered the term "cell lysis fluid" unclear and it has been amended to read "lysed cellular sample" as suggested by the Examiner. One skilled in the art will understand that "lysed cellular sample" and "cell lysis reagent" are not used interchangably.

"Cell lysis reagent" clearly describes a component of a method for lysing cells to extract molecules of interest. The Applicant describes these cell lysis methods as solvent extraction (page 6, line 18-30), acid extraction (page 7, lines 3-24), solid support (page 7, lines 26-30 and page 8, lines 1-11) and detergent methods (pages 8-10). The preferred cell lysis reagent is described on pages 11-12 (lines 25-30 and 1-10, respectively) as a detergent and possible detergents are listed.

Claim 1(ii) is amended herewith to clarify the meaning of the term "mixing the cell lysis fluid with reagents". The term "reagents" has been further defined as "assay reagents" to refer to those reagents used to perform the specific binding assay. Claim 1(ii) is also amended herewith to eliminate redundancy and clarify the meaning of the recited term "for performing a specific binding assay for the analyte". As suggested by the Examiner the language, "to perform a specific binding assay by forming a binding reaction mixture" has been substituted.

Along with the above mentioned amendment to claim 1(ii), one skilled in art will understand the relationship between the "sequestrant" and the "reagents". The term "sequestrant" is defined as a molecule that prevents the cell lysis reagent from adversely affecting the binding reaction between the analyte and the specific binding partner (page 12, lines 11-14). The specification clearly defines the difference between the assay reagents used to perform a specific binding assay in (ii) and the sequestrant in (iii).

Claim 1 is also amended to include step (iv) which incorporates a method for detecting the presence of the specific binding partner-analyte complex as described on page 14 (lines 18-23).

As suggested by the Examiner, claims 2-13 have been amended herewith to include proper antecedent language. The recited "a method as claimed in claim" has been substituted with "the method as claimed in claim...".

The amended claim 1 now provides the proper antecedent support for claim 4. The "said reaction mixture" in amended claim 4 refers to the "reaction mixture" recited in amended claim 1(ii).

Claim 6 is amended herewith to clarify the ambiguity of "multiple assays" by reciting "individual assays are performed in parallel in individual vessels which are wells of a multiwell plate". This multiple assay format is described in the General Assay Conditions beginning on page 21 (line 16). In addition, one skilled in the art would be familiar with setting

up a series of assays with varying conditions on a multiwell plate. The amended claim 6 allows for the distinction between the terms "vessel" and "well". For consistency, claim 7 states "said vessel" to refer to the reaction vessel described in claim 5.

As suggested by the Examiner, claim 13 is amended herewith to utilize more definite and clear language. The parenthetical symbols have been eliminated and the term "concentration is measured of an analyte selected from" has been substituted with "concentration of the analyte is measured and the analyte is selected from a group consisting of...".

The Examiner indicated that claim 14 is of a more narrow scope than the method of claim 1, and therefore is inconsistent and indefinite. The limitations set forth in claim 14 are not presented for utility purposes and it should not be assumed the kit will not function without them. In general, limitations may be introduced into a claim in order to characterize that claim over the prior art. In this case, it is well known that specific binding assays are usually (but not always) performed with one of the assay reagents being labeled with a tracer. Claim 14 is directed to kits for that class of specific binding assays where a tracer is used and as a result no amendment is required.

In view of the aforementioned amendment, the rejection of claims 1-14 under 35 U.S.C. §112 (second paragraph) should properly be withdrawn.

B. The Rejection Under 35 U.S.C. §103(a) Should Be Withdrawn

Claims 1-4, 8-11, and 13-14 stand rejected under 35 U.S.C. §103(a) for allegedly being obvious over Cook (1) in view of Lundin. This rejection should properly be withdrawn because one skilled in the art would not be motivated to combine the use of the scintillation proximity assay of Cook (1) and the sequestrant of Lundin. Furthermore, the combination of these references does not teach the Applicant's invention. It is neither suggested nor apparent from the cited references that the use of a lysis reagent in combination with a sequestrant would allow a highly specific binding reaction to take place. In addition, these references do not indicate that the lysis and specific binding reactions can successfully take place in a single reaction vessel.

Lundin discloses a method of extraction of an intracellular component (ATP, DNA, or RNA), wherein cells are treated with an extractant, and the extract treated with cyclodextrin to neutralize the extractant. Thereafter, ATP may be measured by its ability to

catalyze an enzyme reaction involving firefly luciferase. The components, DNA or RNA, may be used subsequently in enzyme reactions such as PCR. Use of the extracted components in a specific binding assay, such as an immunoassay, is not described.

Cook (1) describes the application of SPA to measure an analyte by means of radioimmunoassay. Briefly, a sample suspected of containing the analyte to be measured is incubated in the presence of radiolabelled analyte and scintillation microspheres coated with a reagent capable of specifically binding the analyte. Bound labeled analyte causes the scintillant microspheres to emit light, whereas unbound labeled analyte will dissipate its β-radiation into the aqueous environment.

The Examiner alleges that one skilled in the art would be motivated to combine these teachings. However, a *prima facie* case of obviousness requires that (1) the prior art suggest to those of ordinary skill in the art that they should make the claimed composition or carry out the claimed process, and that (2) the prior art reveal a reasonable expectation of success in so making the claimed composition or carrying out the claimed process. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438, 1442 (Fed. Cir. 1991).

One skilled in the art would not reasonably be motivated to combine the scintillation proximity assay of Cook (1) in the presence of a sequestrant of Lundin. The use of a lysis reagent in combination with a sequestrant to increase efficacy of a highly specific binding reaction such as that which takes place between an antibody-antigen or receptor-ligand is not suggested by Lundin. Specifically, the problem addressed by the present invention is development of a binding assay for the determination of an intracellular analyte starting from cell culture whereby liberation of the analyte from the cellular environment and the subsequent determination may, if required, be carried out in a "one-pot" process. This problem is solved by the amended claim 1.

Lundin does not disclose the use of the extracted component in a specific binding assay as described in amended claim 1 which includes the step of forming a specific binding partner-analyte complex. Even though Cook (1), does disclose the use of specific binding assays (*e.g.* immunoassays), the document does not motivate one skilled in the art to measure analyte concentrations in cell cultures by means of a specific binding reaction in a "one-pot" process.

Furthermore, combining the methods disclosed in Cook (1) and Lundin will not

teach the Applicant's invention. The Applicant discloses a method of extracting proteins from whole cells in the presence of a sequestrant in order to increase the efficacy of the specific binding reaction. Lundin implicates how to perform cellular extractions in the presence of a sequestrant. While, Cook (1) teaches the method to detect the analyte from cellular fluid. The combination of Lundin and Cook (1) does not teach the extraction of an analyte from whole cell lysis fluid and the detection of this analyte via SPA where a sequestrant enhances the specific binding reaction in the SPA.

These references were both disclosed in the introduction/background of the present application and those cited in Cook (1) disclose methods wherein the measurement is not carried out in the same vessel as the cell lysis step. Even though, it could be alleged that these methods are obvious individually, there is not sufficient reasoning that one skilled in the art would be motivated to combine the two. Individually knowing two methods is not sufficient for rejection under M.P.E.P. §2143.01, there must be a reason to combine the art.

In addition, we would like to draw the Examiner's attention to Brown *et al.* US 5,739,001 which issued as a patent after filing of the present application but which may constitute prior art under 35 US.C. §102(e). Although this is superficially similar to the Applicant's claimed invention, it does not describe a cell lysis step or the use of a sequestrant. On the other hand, Brown *et al.* is believed to be non-relevant to the present invention.

The combination of Lundin and Cook (1) does not teach the extraction of an analyte from a lysed cellular sample and the detection of this analyte via SPA where a sequestrant enhances the SPA specific binding reaction. As a result, the rejection of claims 1-4, 8-11, and 13-14 as being directed to obvious subject matter should be withdrawn.

C. The Rejection Under 35 U.S.C. §103(a) Should Be Withdrawn

The rejection of claims 1-14 under 35 U.S.C. §103(a) for allegedly being obvious over Cook (1) in view of Lundin and in further view of Cook (2) should properly be withdrawn because one skilled in the art would not be motivated to incorporate the multiwell plate of Cook (2) into the present invention. This is because Cook (2) teaches away from the need to disrupt the cells while Applicant's invention is based on lysis of cells and the detection of an intracellular analyte in a single reaction vessel. Furthermore, the present invention teaches the detection of intracellular analyte via an immunoassay and the multiwell plate of Cook (2) does

not allow for the detection of intracellular analyte since it teaches detection of an analyte in intact cells.

Cook (2) discloses an apparatus and a method for studying a cellular process in real time by the use of the apparatus. The apparatus comprises a vessel having a scintillant base which is adapted for the growth and attachment of cells. The method comprises introducing into a fluid suspension of cells a radiolabelled reagent under conditions to cause a portion of the labeled reagent to become associated with or released from the cells so as to study the cellular process.

In this case, one skilled in the art would not be motivated to incorporate the multiwell system described in Cook (2) into the present invention. A primary focus of the present invention is the ability to lyse the cells within the assay dish, then utilize cyclodextrin to neutralize the detergent which will enhance SPA specific binding reactions. The main focus of the multiwell system in Cook (2) is that the scintillant incorporated into the plastic allows the cells to remain intact. As a result, Cook (2) teaches away from the need to disrupt cells in order to measure cellular analytes.

In addition, Cook (2) does not teach the use of an immunoassay for use with the multiwell system. Since Cook (2) focuses on detection in intact cells, it would be impossible to detect via an immunoassay the total intracellular concentration of analyte from an intact cell. Disruption of the cell membrane is necessary to detect the total concentration of analytes described in the application.

The combination of Cook (2) with Lundin and Cook (1) does not teach the use of a multiwell system in which whole cells are cultured, lysed, and assayed in a single reaction vessel. As a result, the rejection of claims 1-14 as being directed to obvious subject matter should be withdrawn.

CONCLUSION

In light of the foregoing amendments and remarks, it is believed that claims 1-14 are in condition for allowance and a notice thereof is respectfully requested. Should the Examiner wish to discuss any further matter of form or substance, she is encouraged to contact undersigned attorney at the telephone number listed below.

Respectfully submitted,

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